U.S. FOOD & DRUG ADMINISTRATION

Biosimilar User Fee Act (BsUFA) III Regulatory Science Pilot Program

ANNUAL REPORT

CONTENTS

Check if this report is Progress or Final Report:

☐ **Progress report** ☒ **Final report**

1. REPORT OVERVIEW[1]

Project Title:	Validation of a non-clinical immunogenicity model
Investigator:	Kristina E. Howard, DVM, Ph.D.
Organization:	DARS/OCP/OTS/CDER
Grant No. (if applicable)	
Project Objective:	This project evaluates the ability of humanized mice to serve as a non-clinical immunogenicity model by evaluating several biological drug products (with clinically moderate to high immunogenicity) alone or in combination in the two most commonly used humanized mouse models.

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Test biological drug products with known clinical immunogenicity, either alone or in combination, and evaluate humanized mice for adaptive immune responses to the products.	All laboratory studies have been completed. For assays completed at time of study, data has been analyzed. For assays, such as anti-drug antibody (ADA) detection, laboratory work is in progress with completion expected by October 2024.	Preliminary results show that immune humanized mice can IgG class switch, and some have shown production of ADA to biologics known to have high rates of immunogenicity.	Some data was presented in a poster at SOT in March 2024, with talks at ACT and the Immunogenicity Summit in 2024. We expect this paper to be submitted for publication before the end of calendar year 2024.
2. Compare the responses of BLT (bone marrow-liver-thymus) humanized mice, that have a human thymus, with CD34 humanized mice, that only have a murine thymus to determine which, if either, can produce adaptive immune responses to biological drug products.	All laboratory studies have been completed. For assays completed at time of study, data has been analyzed. For assays, such as anti-drug antibody (ADA) detection, laboratory work is in progress with completion expected by December 2024.	The data show that while CD34-humanized mice may have the same immune populations as BLT mice (using the same donor), they do not have equivalent immune function as BLT-humanized mice.	Some data was presented in a poster at SOT in March 2024. Additional data will be presented at ACT and the Immunogenicity Summit in 2024. The manuscript is expected to be completed by early 2025.

[1] This section will be used by program for broader research portfolio and regulatory impact analysis by the BsUFA III steering committee.

2. PROGRESS SUMMARY

Project Objective: This project evaluates the ability of humanized mice to serve as a non-clinical immunogenicity model by evaluating several biological drug products (with clinically moderate to high immunogenicity) alone or in combination in the two commonly used humanized mouse models.

This was a single year project that was funded with BsUFA III Regulatory Science Pilot Program funding. At this time all animal studies are complete. We are presently completing the development of anti-drug antibody (ADA) assays that can be used with chimeric serum samples to evaluate for the presence of ADAs to monoclonal antibody and therapeutic protein products. Additional assays using serum samples collected during the study are being evaluated for total immunoglobulin levels by isotype. We have completed analysis of phenotypic and functional data and have begun drafting the manuscript for Aim 1, that we plan to submit for publication in December 2024. Drafting of the manuscript for Aim 2 is expected later this fall, with submission expected in early 2025.

No additional funds are needed to complete publication of the data from these studies.

3. RESEARCH OUTCOMES

The data that have been analyzed thus far show that immune humanized mice have cellular distribution of immune cells that are similar in diversity and representation to that observed in humans. They show the ability to make antibody responses to biological drug products, including class switching and anti-drug antibodies. Through isolation and optimization of lymph node cells, they show the ability to produce cytokines and upregulate activation markers. These responses were found to be most significant in mice that were made with a human thymus and CD34+ hematopoietic stem cells, as compared to mice made using the same donor cells with no human thymus present.

While there are still additional assays to complete, thus far we have good indication that this animal model is sufficiently human to be used for biological/biosimilar drug product research and development.

4. REGULATORY IMPACT

These studies demonstrate the utility of these mice as an animal model for biological drug product testing. Thus far, we have shown that for the assessment of immune function and immunogenicity, the humanized mouse model with human thymus present had more clear and interpretable results as compared to the CD34 humanized mouse model, which may inform the selection of models for different types of studies in the future. Upon completion of the development of planned assays and subsequent data collection, we will show whether or not these animals models can differentiate between products with different known rates of clinical immunogenicity.

While animal studies are not required for biosimilar development nor have animal studies traditionally been conducted, having an available animal model may help with de-risking products prior to transitioning to *in vivo* clinical studies or may help address questions regarding immunogenicity in situations where such *in vivo* clinical studies cannot be performed (e.g., rare diseases with limited patient populations). In addition, these models provide an *in vivo* drug development tool for evaluating functional immune responses for biologics/biosimilars across all facets of drug development.

5. COMMUNICATION AND DISSEMINATION

We have presented preliminary data from these studies at the Society of Toxicology annual meeting in Salt Lake City, UT in March 2024. The posters presented include:

- S. Gharaie, M.K. Chow, R.E. Becker, and K. E. Howard. Assessing phenotype and function of lymph nodes in immune-humanized mice. Poster.

- M.K. Chow, E. Svyatova and K. E. Howard. Development of a proliferation assay for immune-humanized mouse lymphocytes. Poster.

Presentations that will include data from these studies are currently scheduled for the Immunogenicity Summit in October 2024 and the American College of Toxicology meeting in November of 2024.

We are currently preparing the first manuscript, with planned submission this year. The second manuscript will be submitted in the first half of 2025.

These studies are expected to be used as examples in future reviewer training to ensure they are sufficiently familiar with these models to be able to interpret data that could be submitted by sponsors.

6. CHALLENGES

The development of multiple functional assays, and isolation and testing of lymph node cells was challenging, however, no delays were encountered as a result.

7. NEXT STEPS

- Finish running assays and complete data analysis for them.
- Prepare/draft manuscripts for publication.
- Submit manuscripts in Q4 of 2024 and Q2 of 2025.

8. ABBREVIATIONS

This section includes all acronyms used in this document along with a corresponding definition.

ABBREVIATION	DEFINITION
BLT	Bone marrow-liver-thymus
ADA	Anti-drug antibody
IgG	Immunoglobulin G